Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Current Opinion in Insect Science

Abiotic and biotic factors affecting the replication and pathogenicity of bee viruses

Alexander J McMenamin[1,4], Laura M Brutscher[1,4], William Glenny[1,3] and Michelle L Flenniken[1,2,4]

Bees are important pollinators of plants in both agricultural and non-agricultural landscapes. Recent losses of both managed and wild bee species have negative impacts on crop production and ecosystem diversity. Therefore, in order to mitigate bee losses, it is important to identify the factors most responsible. Multiple factors including pathogens, agrochemical exposure, lack of quality forage, and reduced habitat affect bee health. Pathogen prevalence is one factor that has been associated with colony losses. Numerous pathogens infect bees including fungi, protists, bacteria, and viruses, the majority of which are RNA viruses including several that infect multiple bee species. RNA viruses readily infect bees, yet there is limited understanding of their impacts on bee health, particularly in the context of other stressors. Herein we review the influence environmental factors have on the replication and pathogenicity of bee viruses and identify research areas that require further investigation.

Addresses
[1] Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT, USA
[2] Institute on Ecosystems, Montana State University, Bozeman, MT, USA
[3] Department of Ecology, Montana State University, Bozeman, MT, USA
[4] Department of Microbiology and Immunology, Montana State University, Bozeman, MT, USA

Corresponding author: Flenniken, Michelle L (michelle.flenniken@montana.edu)

Current Opinion in Insect Science 2016, **16**:14–21

This review comes from a themed issue on **Vectors and medical and veterinary entomology**

Edited by **Zach N Adelman** and **Kevin Myles**

For a complete overview see the Issue and the Editorial

Available online 26th April 2016

http://dx.doi.org/10.1016/j.cois.2016.04.009

Introduction

Honey bees (*Apis mellifera*), bumble bees (*Bombus* spp.), and other insects play a vital role in ecosystems as plant pollinators. The annual estimated value of crops directly dependent on insect pollination worldwide is $175 billion [1•] and approximately $17-18 billion in both North America and the European Union [2,3]. Wild, native, and managed bee species perform the majority of pollination services in both agricultural and non-agricultural landscapes. Bumble bees are the primary pollinators of some crops (e.g., tomatoes) and augment pollination of other crops [4]. In large-scale crop (e.g., almond, apple, cherry) production honey bees are the primary pollinators, since they forage over large distances and can be maintained in transportable hives. Honey bees were introduced to North America in the early 1600s as a managed species kept by beekeepers primarily for honey production [5]. Today, the majority of US honey bee colonies are maintained by commercial beekeeping operations. Colonies managed by small-scale beekeepers and feral (or unmanaged) colonies make up the remaining population.

High annual losses of managed honey bees and population declines of wild bumble bees are of great concern since bee pollinators are important for plant reproduction and crop production [6,7,8]. In some regions of the US, bumble bees have experienced between 23% and 86% range reduction [7,8] and annual losses of US honey bee colonies have averaged 33% since 2006 (reviewed in [9•]). Several studies have focused on assessing the relationship between colony health and the effects of multiple biotic (e.g., pathogens, bee genetics, and queen longevity) and abiotic factors (e.g., agrochemical exposure, weather, and management practices) [7,10,11,12,13,14]. These studies indicate that pathogens, agrochemical exposure, and lack of quality forage and habitat all contribute to bee losses, though investigating the relative role of these factors is an active area of research. Pathogens, including the microsporidia *Nosema ceranae*, trypanosomatids, viruses, and the ectoparasitic mite *Varroa destructor*, contribute to honey bee colony losses [15,16,17•,18•,19•,20,21,22,23,24,25] (reviewed in [11,26,27,28•,29]), and the microsporidia *Nosema bombi* is associated with declining bumble bee populations in the US [7,8].

The largest class of honey bee infecting pathogens are positive-sense single stranded RNA viruses including: Acute bee paralysis virus (ABPV), Black queen cell virus (BQCV), Israeli acute bee paralysis virus (IAPV), Kashmir bee virus (KBV), Deformed wing virus (DWV), Kakugo virus (KV), Varroa destructor virus-1 (VDV-1), Sacbrood virus (SBV), Slow bee paralysis virus (SBPV), Cloudy wing virus (CWV), Big Sioux River virus (BSRV), Aphid lethal paralysis virus (strain Brookings) (ALPV), Chronic bee paralysis virus (CBPV) (reviewed in [15,17•,28•]), the

Lake Sinai viruses (LSV) [21], and Bee macula-like virus (BeeMLV) [30]. In addition, one double-stranded DNA virus, Apis mellifera filamentous virus (AmFv) has been isolated from honey bees [31]. The majority of bee-infecting viruses were originally discovered and characterized in honey bees, likely since they are the most investigated species. Detection of these viruses in other arthropods indicates that origin of discovery does not necessarily reflect host-range, host–pathogen evolution, or directionality of inter-species transmission (i.e., ABPV, IAPV, DWV, BQCV, SBV, SBPV, LSV and VdMLV) [32••,33••,34,35•,36,37••]. Bee viruses are transmitted both vertically and horizontally [38], including between and among co-foraging wild and managed bee populations [32••,39,40••]. Viruses are also transmitted by *Varroa destructor* mites, which also support replication of a subset of these viruses [41,42,43,44]. Honey bee virus infections may cause deformities, paralysis, death, or remain asymptomatic [15]. The severity of virus infection is influenced by numerous factors that impact bee health, including genetic composition of both host and virus, immune response, synergistic and/or antagonistic pathogenic infections, microbial composition, nutritional status, and agrochemical exposure [15,27,28•,45•,46•,47]. The focus of this review is to highlight recent studies on the abiotic and biotic factors that affect bee virus replication and pathogenicity.

Bee health, nutrition, habitat, and colony management

Bees obtain nutrients from nectar and pollen, and adequate nutrition is important for proper immune system function (reviewed in [48]). Though there have been few quantitative assessments of the relationship between nutritional status and pathogen burden ([49••] and reviewed in [47]), several studies suggest that insufficient protein and low-diversity diets negatively impact bees' ability to defend against pathogens [49••,50,51]. In laboratory-based studies, naturally DWV-infected honey bees that were fed a protein-free sucrose-syrup diet had significantly higher DWV levels compared to bees fed either pollen or a protein-supplement [50]. Intriguingly, the pollen-fed group had reduced DWV virus load by day four of the trial, whereas the protein supplement fed group exhibited reduced virus load several days later [50]. While an adequate amount of protein is important, a diverse pollen diet, as opposed to monofloral pollen or additional protein, enhanced adult bee immunocompetence (i.e., haemocyte concentration, fat body mass, and phenoloxidase and glucose oxidase activities) [49••]. Together these studies suggest that while protein is important, the source of this protein is also critical to proper immune function. Similarly, bees fed honey, which consists of 30–45% fructose, 24–40% glucose, 0.1–4.8% disaccharides including sucrose, and minute amounts of micronutrients and amino acids, exhibited increased expression in more genes involved in detoxification, immunity, aromatic amino acid metabolism, and oxidation and reduction, as compared to bees fed either sucrose or high fructose corn syrup [51,52]. Together, these studies indicate that proper nutrition (i.e., adequate protein and carbohydrates) and natural and diverse food sources (i.e., nectar and pollen) enhance bee immune function. However, the mechanisms and gene regulatory pathways involved in nutrition-dependent immunocompetence require further characterization. Future studies should employ both cage-studies, which provide a well-controlled environment to investigate individual bee responses and facilitate standardization of multiple variables (e.g., pathogen dose), and colony level studies. A more thorough understanding of the role of diet on bee health is important, as it is common for beekeepers to provide supplemental feed when natural sources are scarce. Overall, these studies indicate that managing landscapes to enhance floral, and therefore nutritional diversity will benefit the health of both managed and wild bee populations.

While floral resources are essential to bee health, flowers also serve as a hub for pathogen transmission and agrochemical exposure [32••,33••,40••]. The most well documented intra- and inter-species transmissible bee pathogens are RNA viruses [32••,33••,39,53••,54,55••]. Transmission of these viruses is thought to be associated with bee foraging activities, as BQCV, SBV, and DWV have been detected in honey bee collected pollen [32••,40••]. In addition, inter-species transmission was demonstrated experimentally in greenhouse studies in which IAPV was transmitted from honey bees to bumble bees and vice versa [32••]. Phylogenetic analyses of virus genome sequences (i.e., BQCV, DWV, and IAPV) obtained from foraging honey bees, pollen pellets, and non-*Apis* hymenopteran, including solitary bees, wasps, and bumble bees, did not cluster by host, providing further evidence of inter-species transmission [32••]. In addition, IAPV was detected in non-*Apis* hymenopteran species collected from sites near IAPV-infected honey bee colonies, whereas wild hymenopterans obtained from areas proximal to honey bees that were not infected with IAPV were also IAPV-negative [32••]. Likewise, recent evaluation of the viruses associated with sympatric honey bee and bumble bee populations in Great Britain and the Isle of Man indicated they were infected with similar strains of DWV and VDV [39], and BQCV, DWV, ABPV, SBPV, and SBV were detected in both honey bees and bumble bees in the same geographic area, though viral prevalence and abundance varied by species [33••]. Based on modeling data, it was suggested that the directionality of DWV transmission was from honey bees to bumble bees, since DWV was more prevalent and abundant in honey bees than in bumble bees where ranges overlapped [39]. This relationship was reversed for ABPV and SBV, which were more prevalent in bumble bees than in honey bees where ranges overlapped [33••]. Although viruses are shared between honey bees and bumble bees, there have been very few studies that have investigated the role of viruses on bumble bee health, as most efforts have focused on the role of eukaryotic pathogens on bumble bee health [53••,56,57,58,59]. Additional epidemiologic studies are required to better understand pathogen transmission between and within wild and managed bee populations, since the dynamics of transmission will likely vary across different geographies and be influenced by the local abundance of particular bee species, pathogen prevalence, and anthropogenic factors including land use [48,60•] and agrochemical exposure [61••,62••]. Proximity to urbanization and colony management have been linked to increased pathogen pressure on honey bees [60•]. A study of feral and non-commercially managed honey bee colonies across an urbanization gradient determined that feral bees were more immunocompetent (as indicated by approximately two-fold increased expression of four immune genes after challenge) than managed bees, that urbanization positively correlated with greater *Nosema ceranae* and BQCV prevalence, and that management was positively correlated with higher prevalence of both *Nosema apis* and *Nosema ceranae* [60•].

Impact of agrochemical exposure on virus replication and pathogenesis

Bee health is influenced by a variety of environmental factors including exposure to agrochemicals. Agrochemicals, including pesticides, herbicides, and fungicides are used widely across a range of landscapes (e.g., agricultural, non-agricultural, wild, managed, and residential), as well as within managed honey bee colonies. Agrochemical exposure sometimes results in acute bee losses, as well as sublethal toxicity, therefore there is much concern regarding the role of pesticides, particularly neonicotinoids, in bee declines (reviewed in [62••,63,64]). Although, the latest insecticide formulations may pose less of a threat to bee health as compared to previous formulations [62••,64,65]. Compared to other insects, honey bees have a reduced repertoire of genes involved in detoxification [66], and at least one study indicated that bees prefer neonicotinoid-containing food [67•]; these studies underscore the importance of further examining the risks associated with agrochemical exposure. Many studies have found that insecticides, including neonicotinoids, negatively impact bee health ([18•,68,69•,70,71,72•,73••,74,75,76] and reviewed in [62••]). However, several studies determined that typical field exposure levels are below known toxicity thresholds [77,78,79]; specifically, oral administration of imidacloprid at 5 ppb [77,78] or contact with thiacloprid at doses below 6 μg/bee (approximately 50 ppm) [80] had no observable negative impact.

The majority of studies investigating the effects of agrochemicals on bee health have focused on neonicotinoids (reviewed in [62••]). Several studies suggest that exposure to these chemicals increases pathogen abundance [18•,76,81]. Specifically, full sized honey bee colonies exposed to imidacloprid (2 or 20 ppb in pollen patties) had greater levels of *Nosema ceranae* than unexposed colonies [18•]. Likewise, exposing bees to imidacloprid and clothianidin topically (0, 10, 20, and 30 ng per bee, which corresponds to approximately 83, 167, and 450 ppm) and orally (0.1, 1.0, and 10 ppb) resulted in a dose-dependent increase of DWV levels [73••]. Similarly, sublethal, though not necessarily field-relevant, doses of thiacloprid (0.1 ppm in larval food) increased BQCV titers and larval mortality [76]. This indicates that agrochemical exposure and viral infection synergistically harm larvae, though negative impacts were not observed in adults [76].

There are numerous other (non-neonicotinoid) agrochemicals that are utilized in both agricultural and non-agricultural settings that have received less attention and scientific investigation [82], though they may impact pathogen abundance and bee health. Chlorpyrifos, an organophosphate, and Pristine®, a fungicide composed of boscalid and pyraclostrobin used during almond bloom, negatively affected queen health [70]. Chlorpyrifos decreased queen emergence and increased DWV abundance, but not prevalence, in queens relative to the nurse bees tending them [70]. Colonies treated with chlorpyrifos and Pristine® had decreased queen emergence, but viral prevalence or abundance was not affected relative to chlorpyrifos alone [47]. In contrast, reduced queen emergence was not found when colonies in isolated swarm boxes were fed pollen treated with Pristine® or Pristine® with an adjuvant, whereas colonies treated with diflubenzuron, an insect growth regulator, had a significant reduction on queen survival [83].

In addition to agrochemical exposure from foraging (i.e., nectar and pollen) and via food sources (i.e., bee bread and royal jelly), honey bees are also exposed to agrochemicals within the colony (e.g., antibiotics and miticides). Beekeepers routinely utilize the fungicide Fumagillan-B® to reduce levels of *Nosema apis* and *Nosema ceranae*, and acaricides (e.g., tau-fluvalinate, thymol, coumaphos, formic acid, and amitraz) to reduce *Varroa destructor* mite infestation [61••,84]. While *Varroa* is one of many biotic factors contributing to colony losses [85], high *Varroa* levels, above the threshold of >3 mites per 100 bees [86], are associated with increased pathogen load [17•,87,88••] (reviewed in [89]). Furthermore, mites serve as a mechanism for pathogen transmission between colonies (reviewed in [90]). Unfortunately, some research suggests that acaricides may reduce honey bee immunocompetence [91]. Bees with compromised immune responses would be expected to harbor greater pathogen loads, though acaricide treated bees exhibited variable levels of pathogens [88••,91]. In contrast, acaricide treatment did not affect pathogen loads in colonies with low mite pressure [91]. However, bees obtained from colonies that were treated with thymol and coumaphos exhibited significantly reduced expression of two immune genes (DSC37 and BASK) [91]. Interestingly, tau-fluvalinate application resulted in increased DWV levels in adult honey bees and *Varroa*, BQCV in mite-infested pupae, and SBV in pupae not infested with mites [88••]. Importantly, tau-fluvalinate treated colonies showed long-term reduction in DWV and DWV-associated symptoms, and thus demonstrated that proper acaricide use is important, and may be effective in controlling DWV levels [88••]. The relationships between mite levels, acaricide exposure, viral abundance, and bee immune gene expression are complex and variable. However, in one study, increased expression of honey bee immune genes (*relish*, *PGRP-S1*, *hymenoptaecin*, *apidaecin*, *defensin*, and *PPOAct*) corresponded to decreased mite reproduction [92••]. In addition, this study found no evidence that *Varroa* negatively impacted honey bee immunocompetence [92••]. The majority of colonies in North America are infested with *Varroa* mites [90] and most managed honey bees in North America are continuously exposed to acaricides in wax [61••]. Therefore, a better understanding of the impact of these stressors on bee health is an important area of ongoing research.

For many agrochemical formulations a lethal dose 50 (LD_{50}) and/or exposure threshold for bees at various stages of development is not known and even less information is available regarding synergistic interactions of these chemicals in bees [62••,93,94]. Regardless, research and public opinion have resulted in bans on the use of clothianidin, thiamethoxam, and imidacloprid in the EU [95] and have resulted in additional US EPA regulations on new registrations for neonicotinoids [96]. The evidence to date suggests that bee colony losses are not solely dependent upon agrochemicals, but are likely a result of a combination of factors. Agrochemicals can pose problems, but they are often required for large-scale production of agricultural crops, and their proper use as part of an integrated pest management strategy (IPM) often results in low to no levels of exposure in field settings [77,78,79]. These studies and increased prophylactic usage of neonicotinoids (i.e., as treated seeds with no other purchase options), underscore the importance of continued research on the effects of agrochemicals on bee health. In addition, loss of forage due to herbicide use in many landscapes impacts the availability of quality forage for all bee species (reviewed in [78]).

Bee microbiome

Bee-associated microbes are not limited to pathogens, but also include commensal microbes ([97,98] and reviewed in [46•]). The best characterized commensal microbes of bees are honey bee gut associated bacteria, including eight bacterial phylotypes predominantly in the *Proteobacteria*, *Firmicutes*, and *Actinobacteria* phyla ([99,100] and reviewed in [46•]). The relationship between the gut microbiome and viruses has been characterized in mammals (reviewed in [101]) and in solitary insects, including fruit flies and mosquitoes. In fruit flies and mosquito spp., several strains of the bacteria *Wolbachia* reduce RNA virus replication and plasmodium infection [102,103]. *Wolbachia* 16S rRNA sequences have been detected in different subspecies of *Apis mellifera* samples from southern Africa [104] and Germany [105], and in five species of European bumble bees [110], but the potential influence of *Wolbachia* on virus infections in bees has not been studied. Recent findings suggest that *Parasaccharibacter apiumin* may improve larval survival [106], and enhance defense against *Nosema* [107], but the potential effects of this bacteria on virus replication is not known. Bee microbiome research has primarily focused on the benefits of these microbes to bee health, but not all bee-associated bacteria are beneficial; some may be opportunistic pathogens (e.g., *F. perrara*) [108,109], whereas others (i.e., *Paenibacillus larvae* and *Melissococcus plutonius*) are pathogenic. The relationship between the bee bacteriome and virome, as well as the effects of both on bee health require further characterization.

Summary

Bee pollinators inhabit a range of environments including wild, agricultural, and urban landscapes. In these diverse settings, multiple factors including pathogens, nutrient availability, agrochemical exposure, and the microbiome converge to affect bee health. These factors affect bee immunocompetence and virus replication and pathogenicity. Furthermore, land and pollinator management practices impact bee health and may result in increased pathogen pressure on bees. Managing landscapes to enhance floral diversity will benefit the health of both commercial and wild bee populations. Floral resources are not only important to bee health, but also serve as sites of pathogen transmission and agrochemical exposure. Agrochemicals, including those used within honey bee colonies, seem to impact disease severity, though the processes involved require further elucidation. Lastly, the emerging field of insect microbiome research presents exciting avenues of inquiry, including how the bacteriome and virome interact at the host-level. Better understanding of bee biology, the factors that influence bee health, pathogen transmission, and immune mechanisms will result in the development of management practices that support pollinator health.

Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgements

The Flenniken Laboratory is supported by the United States Department of Agriculture National Institute of Food and Agriculture, Agriculture and Food Research Initiative (USDA-NIFA-AFRI) Program, Montana Department of Agriculture Specialty Crop Block Grant Program, the National Institutes of Health IDeA Program COBRE grant GM110732, National Science Foundation EPSCoR NSF-IIA-1443108, Hatch Multistate

Funding (NC-1173), Project *Apis m.*, the Montana State Beekeepers Association, Montana State University, and the Montana State University Agricultural Experiment Station. Laura M. Brutscher is supported by the Project *Apis m.*-Costco Honey Bee Biology Fellowship. We would like to thank members of the Flenniken laboratory (Katie Daughenbaugh, Emma Garcia, Jenna Severson, Cayley Faurot-Daniels, and Elisa Boyd) for reviewing this manuscript prior to publication.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Gallai N, Salles J-M, Settele J, Vaissière BE: **Economic valuation of the vulnerability of world agriculture confronted with pollinator decline**. *Ecol Econ* 2009, **68**:810-821.
This paper calculates the economic value of insect-dependent pollination services from 1992 to 2009, broken down into *Apis*, non-*Apis* and alfalfa leaf-cutter bee pollination.

2. Calderone NW: **Insect pollinated crops, insect pollinators and US agriculture: trend analysis of aggregate data for the period 1992-2009**. *PLoS ONE* 2012, **7**:e37235.

3. Leonhardt SD, Gallai N, Alejandro Garibaldi L, Kuhlmann M, Klein A-M: **Economic gain, stability of pollination and bee diversity decrease from southern to northern Europe**. *Basic Appl Ecol* 2013, **14**:461-471.

4. Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalheiro LG, Harder LD, Afik O *et al.*: **Wild pollinators enhance fruit set of crops regardless of honey bee abundance**. *Science* 2013, **339**:1608-1611.

5. Sheppard WS: **A History of the Introduction of Honey Bee Races Into the United-States**. *Am Bee J* 1989, **129**:617-619.

6. Potts SG, Roberts S, Dean R, Marris G, Brown M, Jones R, Neumann P, Settele J: **Declines of managed honey bees and beekeepers in Europe**. *J Apic Res* 2010, **49**:15-22.

7. Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswold TL: **Patterns of widespread decline in North American bumble bees**. *Proc Natl Acad Sci USA* 2011, **108**:662-667.

8. Goulson D, Nicholls E, Botías C, Rotheray EL: **Bee declines driven by combined stress from parasites, pesticides, and lack of flowers**. *Science* 2015, **347**:1255957.

9. • Lee KV, Steinhauer N, Rennich K, Wilson ME, Tarpy DR, Caron DM, Rose R, Delaplane KS, Baylis K, Lengerich EJ *et al.*: **A national survey of managed honey bee 2013-2014 annual colony losses in the USA**. *Apidologie* 2015, **46**:292-305.
This paper described the most recent results from an ongoing annual survey of colony losses in the USA.

10. Burkle LA, Marlin JC, Knight TM: **Plant-pollinator interactions over 120 years: loss of species. Co-occurrence and function**. *Science* 2013 http://dx.doi.org/10.1126/science.1232728.

11. Nazzi F, Pennacchio F: **Disentangling multiple interactions in the hive ecosystem**. *Trends Parasitol.* 2014, **30**:556-561.

12. Pettis JS, Delaplane KS: **Coordinated responses to honey bee decline in the USA**. *Apidologie* 2010, **41**:256-263.

13. van der Zee R, Pisa L, Andonov S, Brodschneider R, Charriere J-D, Chlebo R, Coffey MF, Crailsheim K, Dahle B, Gajda A *et al.*: **Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008-9 and 2009-10**. *J Apicult Res* 2012, **51**:110-114.

14. Steinhauer NA, Rennich K, Lee K, Pettis J, Tarpy DR, Rangel J, Caron D, Sagili RR, Skinner JA, Wilson ME *et al.*: *Colony Loss 2014-2015: Preliminary Results [Internet]*. 2015:. beeinformed.org.

15. Chen YP, Siede R: **Honey Bee Viruses.Advances in Virus Research**. Elsevier; 2007:33-80.

16. Evans JD, Schwarz RS: **Bees brought to their knees: microbes affecting honey bee health**. *Trends Microbiol* 2011, **19**:614-620.

17. • Genersch E, Aubert M: **Emerging and re-emerging viruses of the honey bee (*Apis mellifera* L.)**. *Vet Res* 2010, **41**:54.
This paper provides a comprehensive review of the interaction of two virus groups, ABPV/KBV/IAPV and DWV/VDV, with *Varroa destructor* mites as well as the synergistic effects of mites and viruses on honey bee health.

18. • Pettis JS, vanEngelsdorp D, Johnson J, Dively G: **Pesticide exposure in honey bees results in increased levels of the gut pathogen Nosema**. *Naturwissenschaften* 2012, **99**:153-158.
This study demonstrated an interaction between sublethal exposure to imidacloprid at the colony level and degree of *Nosema* infection in individual bees. It is a clear demonstration of synergistic negative impacts of both agrochemical exposure and pathogenic infections on honey bee health.

19. • Cornman RS, Tarpy DR, Chen Y, Jeffreys L, Lopez D, Pettis JS, vanEngelsdorp D, Evans JD: **Pathogen webs in collapsing honey bee colonies**. *PLoS ONE* 2012, **7**:e43562.
This paper gives a thorough analysis of multiple infections in collapsing colonies as compared healthy colonies.

20. Ravoet J, Maharramov J, Meeus I, De Smet L, Wenseleers T, Smagghe G, de Graaf DC: **Comprehensive bee pathogen screening in Belgium reveals *Crithidia mellificae* as a new contributory factor to winter mortality**. *PLoS ONE* 2013, **8**:72443.

21. Daughenbaugh KF, Martin M, Brutscher LM, Cavigli I, Garcia E, Lavin M, Flenniken ML: **Honey Bee Infecting Lake Sinai Viruses**. *Viruses* 2015, **7**:3285-3309.

22. Hou C, Rivkin H, Slabezki Y, Chejanovsky N: **Dynamics of the presence of Israeli Acute Paralysis Virus in honey bee colonies with colony collapse disorder**. *Viruses* 2014, **6**:2012-2027.

23. Highfield AC, Nagar El A, Mackinder LCM, Noel LM-LJ, Hall MJ, Martin SJ, Schroeder DC: **Deformed wing virus implicated in overwintering honeybee colony losses**. *Appl Environ Microbiol* 2009, **75**:7212-7220.

24. Genersch E, Ohe von der W, Kaatz H, Schroeder A, Otten C, Büchler R, Berg S, Ritter W, Mühlen W, Gisder S *et al.*: **The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies**. *Apidologie* 2010, **41**:332-352.

25. Higes M, Martin-Hernandez R, Martinez-Salvador A, Garrido-Bailon E, Virginia Gonzalez-Porto A, Meana A, Luis Bernal J, Jesus del Nozal M, Bernal J: **A preliminary study of the epidemiological factors related to honey bee colony loss in Spain**. *Environ Microbiol Rep* 2010, **2**:243-250.

26. Barron AB: **Death of the bee hive: understanding the failure of an insect society**. *Curr Opin Insect Sci* 2015. [no volume].

27. McMenamin AJ, Genersch E: **Honey bee colony losses and associated viruses**. *Curr Opin Insect Sci* 2015 http://dx.doi.org/10.1016/j.cois.2015.01.015.

28. • Brutscher LM, Daughenbaugh KF, Flenniken ML: **Antiviral defense mechanisms in honey bees**. *Curr Opin Insect Sci* 2015 http://dx.doi.org/10.1016/j.cois.2015.04.016.
This review provides a comprehensive overview of honey bee antiviral defense mechanisms.

29. vanEngelsdorp D, Tarpy DR, Lengerich EJ, Pettis JS: **Idiopathic brood disease syndrome and queen events as precursors of colony mortality in migratory beekeeping operations in the eastern United States**. *Prev Vet Med* 2013, **108**:225-233.

30. de Miranda JR, Cornman RS, Evans JD, Semberg E, Haddad N, Neumann P, Gauthier L: **Genome characterization, prevalence and distribution of a macula-like virus from *Apis mellifera* and Varroa destructor**. *Viruses* 2015, **7**:3586-3602.

31. Gauthier L, Cornman S, Hartmann U, Cousserans F, Evans JD, de Miranda JR, Neumann P: **The *Apis mellifera* filamentous virus genome**. *Viruses* 2015, **7**:3798-3815.

32. •• Singh R, Levitt AL, Rajotte EG, Holmes EC, Ostiguy N, vanEngelsdorp D, Lipkin WI, Depamphilis CW, Toth AL, Cox-Foster DL: **RNA viruses in hymenopteran pollinators: evidence of inter-Taxa virus transmission via pollen and potential impact on non-Apis hymenopteran species**. *PLoS ONE* 2010, **5**:e14357.

This paper presented some of the first evidence that RNA viruses infect multiple bee species in natural systems and demonstrated inter-species transmission between honey bees and bumble bees in greenhouse studies.

33. •• McMahon DP, Fürst MA, Caspar J, Theodorou P, Brown MJF, Paxton RJ: **A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees**. *J Anim Ecol* 2015 http://dx.doi.org/10.1111/1365-2656.12345.
This study was one of the first large-scale epidemiological surveys of viruses co-occurring in sympatric honey bee and bumble bee populations in the UK.

34. Levitt AL, Singh R, Cox-Foster DL, Rajotte E, Hoover K, Ostiguy N, Holmes EC: **Cross-species transmission of honey bee viruses in associated arthropods**. *Virus Res* 2013, **176**:232-240.

35. • Li J, Peng W, Wu J, Strange JP, Boncristiani H, Chen Y: **Cross-species infection of deformed wing virus poses a new threat to pollinator conservation**. *J Econ Entomol* 2011, **104**:732-739.
This was one of the first studies to examine tissue-specific DWV levels and replication in field collected bumble bees.

36. Peng W, Li J, Boncristiani H, Strange JP, Hamilton M, Chen Y: **Host range expansion of honey bee Black Queen Cell Virus in the bumble bee, *Bombus huntii***. *Apidologie* 2011, **42**:650-658.

37. •• Parmentier L, Smagghe G, de Graaf DC, Meeus I: **Varroa destructor Macula-like virus, Lake Sinai virus and other new RNA viruses in wild bumblebee hosts (*Bombus pascuorum*, *Bombus lapidarius* and *Bombus pratorum*)**. *J Invertebr Pathol* 2015 http://dx.doi.org/10.1016/j.jip.2015.12.003.
This study was the first to survey the presence of VMLV, LSV, and other RNA viruses in three wild bumble bee species. Furthermore, they determined that populations near apiaries were more likely to be infected, and that LSV infection may either increase the likelihood of a second infection or be a predictor of co-infection.

38. Chen YP, Pettis JS, Collins A, Feldlaufer MF: **Prevalence and transmission of honeybee viruses**. *Appl Environ Microbiol* 2006, **72**:606-611.

39. Fürst MA, McMahon DP, Osborne JL, Paxton RJ, Brown MJF: **Disease associations between honeybees and bumblebees as a threat to wild pollinators**. *Nature* 2014, **506**:364-366.

40. •• Mazzei M, Carrozza ML, Luisi E, Forzan M, Giusti M, Sagona S, Tolari F, Felicioli A: **Infectivity of DWV associated to flower pollen: experimental evidence of a horizontal transmission route**. *PLoS ONE* 2014, **9**:e113448.
This study presented evidence that field-collected pollen contained infective DWV, and that the solitary bee *Osmia cornuta* can serve as a DWV host.

41. de Miranda JR, Dainat B, Locke B, Cordoni G, Berthoud H, Gauthier L, Neumann P, Budge GE, Ball BV, Stoltz DB: **Genetic characterization of slow bee paralysis virus of the honeybee (*Apis mellifera* L.)**. *J Gen Virol* 2010, **91**:2524-2530.

42. Boncristiani HF, Di Prisco G, Pettis JS (null), Hamilton M, Chen YP: **Molecular approaches to the analysis of deformed wing virus replication and pathogenesis in the honey bee, *Apis mellifera***. *Virol J* 2009, **6**:221.

43. Chen Y, Pettis J, Evans J, Kramer M, Fedlaufer M: **Transmission of Kashmir bee virus by the ectoparasitic mite Varroa destructor**. *Apidologie* 2004, **35**:441-448.

44. Shen M, Cui L, Ostiguy N, Cox-Foster D: **Intricate transmission routes and interactions between picorna-like viruses (Kashmir bee virus and sacbrood virus) with the honeybee host and the parasitic varroa mite**. *J Gen Virol* 2005, **86**:2281-2289.

45. • Martin SJ, Highfield AC, Brettell L, Villalobos EM, Budge GE, Powell M, Nikaido S, Schroeder DC: **Global honey bee viral landscape altered by a parasitic mite**. *Science* 2012, **336**:1304-1306.
This paper showed evidence that the viral vector *Varroa destructor* influenced DWV strain prevalence in honey bees.

46. • Moran NA: **Genomics of the honey bee microbiome**. *Curr Opin Insect Sci* 2015, **10**:22-28.
Excellent review of the honey bee microbiome research.

47. DeGrandi-Hoffman G, Chen Y: **Nutrition, immunity and viral infections in honey bees**. *Curr Opin Insect Sci* 2015, **10**:170-176.

48. Vaudo AD, Tooker JF, Grozinger CM, Patch HM: **Bee nutrition and floral resource restoration**. *Curr Opin Insect Sci* 2015, **10**:133-141.
This paper is a great review of bee nutritional requirements and suggests how to use this knowledge in habitat restoration.

49. •• Alaux C, Ducloz F, Crauser D, Le Conte Y: **Diet effects on honeybee immunocompetence**. *Biol Lett* 2010, **6**:562-565.
This study demonstrated that bee health is improved by diets that include diverse pollen sources; bees fed monofloral pollen sources had reduced levels of immune gene expression, as compared to bees fed pollen from multiple sources.

50. DeGrandi-Hoffman G, Chen Y, Huang E, Huang MH: **The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.)**. *J Insect Physiol* 2010, **56**:1184-1191.

51. Wheeler MM, Robinson GE: **Diet-dependent gene expression in honey bees: honey vs. sucrose or high fructose corn syrup**. *Sci Rep* 2014, **4**:5726.
This study examined the effect of diet on gene expression and provided a basis for understanding the mechanisms of nutritional factors on host physiology.

52. Mao W, Schuler MA, Berenbaum MR: **Honey constituents up-regulate detoxification and immunity genes in the western honey bee *Apis mellifera***. *Proc Natl Acad Sci USA* 2013 http://dx.doi.org/10.1073/pnas.1303884110.

53. •• Graystock P, Meeus I, Smagghe G, Goulson D, Hughes WOH: **The effects of single and mixed infections of *Apicystis bombi* and deformed wing virus in *Bombus terrestris***. *Parasitology* 2015 http://dx.doi.org/10.1017/S0031182015001614.
This study examined the pathophysiological effects of mixed infection in bumble bees.

54. Genersch E, Yue C, Fries I, de Miranda JR: **Detection of Deformed wing virus, a honey bee viral pathogen, in bumble bees (*Bombus terrestris* and *Bombus pascuorum*) with wing deformities**. *J Invertebr Pathol* 2006, **91**:61-63.

55. •• Ravoet J, De Smet L, Meeus I, Smagghe G, Wenseleers T, de Graaf DC: **Widespread occurrence of honey bee pathogens in solitary bees**. *J Invertebr Pathol* 2014, **122**:55-58.
This study examined pathogen occurrence in European solitary bees, and was the first to report detection pathogens that were previously primarily associated with honey bees in solitary bee species.

56. Colla SR, Otterstatter MC, Gegear RJ, Thomson JD: **Plight of the bumble bee: pathogen spillover from commercial to wild populations**. *Biol Conserv* 2006, **129**:461-467.

57. Barribeau SM, Schmid-Hempel P: **Qualitatively different immune response of the bumblebee host, *Bombus terrestris*, to infection by different genotypes of the trypanosome gut parasite, *Crithidia bombi***. *Infect Genet Evol* 2013, **20**:249-256.

58. Manley R, Boots M, Wilfert L: **Emerging viral disease risk to pollinating insects: ecological, evolutionary and anthropogenic factors**. *J Appl Ecol* 2015 http://dx.doi.org/10.1111/1365-2664.12385.

59. Otterstatter MC, Thomson JD: **Does pathogen spillover from commercially reared bumble bees threaten wild pollinators?** *PLoS ONE* 2008, **3**:e2771.

60. • Youngsteadt E, Appler RH, López-Uribe MM, Tarpy DR, Frank SD: **Urbanization increases pathogen pressure on feral and managed honey bees**. *PLoS ONE* 2015, **10**:e0142031.
This study provided important information of the effects of urban landscapes and management on bee immunocompetence.

61. •• Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, vanEngelsdorp D, Pettis JS: **High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health**. *PLoS ONE* 2010, **5**:e9754.
This study was the first comprehensive examination of the agrochemicals associated with North American honey bee colonies; they reported frequent detection of numerous chemicals associated with bee colonies.

62. •• Johnson RM: **Honey bee toxicology**. *Annu Rev Entomol* 2014 http://dx.doi.org/10.1146/annurev-ento-011613-162005.
This is a comprehensive review of the affects of toxins on bees, including phytochemicals and agrochemicals.

63. Johnson RM, Ellis MD, Mullin CA, Frazier M: **Pesticides and honey bee toxicity — USA**. *Apidologie* 2010, **41**:312-331.

64. Krupke CH, Long EY: **Intersections between neonicotinoid seed treatments and honey bees**. *Curr Opin Insect Sci* 2015, **10**:8-13.

65. Hardstone MC, Scott JG: **Is *Apis mellifera* more sensitive to insecticides than other insects?** *Pest Manage Sci* 2010 http://dx.doi.org/10.1002/ps.2001.

66. Berenbaum MR, Johnson RM: **Xenobiotic detoxification pathways in honey bees**. *Curr Opin Insect Sci* 2015.

67. • Kessler SC, Tiedeken EJ, Simcock KL, Derveau S, Mitchell J, Softley S, Stout JC, Wright GA: **Bees prefer foods containing neonicotinoid pesticides**. *Nature* 2015, **521**:74-76.
This study demonstrated that bees prefer neonicotinoid contaminated food sources and, thus, emphasized the need for additional research focused on determining the affects of these and other agrochemical on bee health, particularly the potential sublethal and behavioral effects.

68. Wu JY, Smart MD, Anelli CM, Sheppard WS: **Honey bees (*Apis mellifera*) reared in brood combs containing high levels of pesticide residues exhibit increased susceptibility to Nosema (Microsporidia) infection**. *J Invertebr Pathol* 2012, **109**:326-329.

69. • Wu JY, Anelli CM, Sheppard WS: **Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*Apis mellifera*) development and longevity**. *PLoS ONE* 2011, **6**:e14720.
This study demonstrated that worker bees exposed to pesticide contamination experienced negative sublethal effects.

70. DeGrandi-Hoffman G, Chen Y, Simonds R: **The effects of pesticides on queen rearing and virus titers in honey bees (*Apis mellifera* L.)**. *Insects* 2013, **3**:601-615.

71. Blacquière T, Smagghe G, van Gestel CAM, Mommaerts V: **Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment**. *Ecotoxicology* 2012 http://dx.doi.org/10.1007/s10646-012-0863-x.

72. • Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K: **Multiple routes of pesticide exposure for honey bees living near agricultural fields**. *PLoS ONE* 2012, **7**:e29268.
This study describes exposure of bees to agrochemicals via multiple routes throughout the growing season.

73. •• Di Prisco G, Cavaliere V, Annoscia D, Varricchio P, Caprio E, Nazzi F, Gargiulo G, Pennacchio F: **Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees**. *Proc Natl Acad Sci USA* 2013 http://dx.doi.org/10.1073/pnas.1314923110.
This study was one of the first to show a clear link between pesticide exposure and increased pathogen load. Furthermore, it was the first study to propose a mechanism for this effect and investigated it in both fruit flies and honey bees.

74. Henry M, Béguin M, Requier F, Rollin O, Odoux J-F, Aupinel P, Aptel J, Tchamitchian S, Decourtye A: **A common pesticide decreases foraging success and survival in honey bees**. *Science* 2012, **336**:348-350.

75. Gill RJ, Ramos-Rodriguez O, Raine NE: **Combined pesticide exposure severely affects individual- and colony-level traits in bees**. *Nature* 2012 http://dx.doi.org/10.1038/nature11585.

76. Doublet V, Labarussias M, de Miranda JR, Moritz RFA, Paxton RJ: **Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle**. *Environ Microbiol* 2014 http://dx.doi.org/10.1111/1462-2920.12426.

77. Faucon J-P, Auri res CM, Drajnudel P, Mathieu L, Ribi re M, Martel A-C, Zeggane S, Chauzat M-P, Aubert MF: **Experimental study on the toxicity of imidacloprid given in syrup to honey bee (*Apis mellifera*) colonies**. *Pest Manage Sci* 2005, **61**:111-125.

78. Dively GP, Embrey MS, Kamel A, Hawthorne DJ, Pettis JS: **Assessment of chronic sublethal effects of imidacloprid on honey bee colony health**. *PLoS ONE* 2015, **10**:e0118748.
This study presented data from a three year study on the sublethal effects of a neonicotinoid in colony-based studies and found a dose-dependent effect, though field relevant doses had a negative effect.

79. Lawrence TJ, Culbert EM, Felsot AS, Hebert VR, Sheppard WS: **Survey and risk assessment of *Apis mellifera* (Hymenoptera: Apidae) exposure to neonicotinoid pesticides in urban, rural, and agricultural settings**. *J Econ Entomol* 2016 http://dx.doi.org/10.1093/jee/tov397.

80. Schmuck R, Stadler T, Schmidt H-W: **Field relevance of a synergistic effect observed in the laboratory between an EBI fungicide and a chloronicotinyl insecticide in the honeybee (*Apis mellifera* L, Hymenoptera)**. *Pest Manage Sci* 2003, **59**:279-286.

81. Di Prisco G, Cavaliere V, Annoscia D, Varricchio P, Caprio E, Nazzi F, Gargiulo G, Pennacchio F: **Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees**. *Proc Natl Acad Sci USA* 2013, **110**:18466-18471.

82. Pohanish RP: *Sittig's Handbook of Pesticides and Agricultural Chemicals*. William Andrew Pub; 2014.

83. Johnson RM, Percel EG: **Effect of a fungicide and spray adjuvant on queen-rearing success in honey bees (Hymenoptera: Apidae)**. *J Econ Entomol* 2013, **106**:1952-1957.

84. Coalition HBH: *Tools for Varroa Management [Internet]*. The Keystone Policy Center on behalf of The Honey Bee Health Coalition; 2015.

85. vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen Y *et al.*: **Colony collapse disorder: a descriptive study**. *PLoS ONE* 2009, **4**:e6481.

86. Rose R, Pettis J, vanEngelsdorp D: **A US national survey of honey-bee pests and diseases**. Internet, www.oie.int.

87. Francis RM, Nielsen SL, Kryger P: **Varroa-virus interaction in collapsing honey bee colonies**. *PLoS ONE* 2013, **8**:e57540.

88. •• Locke B, Forsgren E, Fries I, de Miranda JR: **Acaricide treatment affects viral dynamics in Varroa destructor-infested honey bee colonies via both host physiology and mite control**. *Appl Environ Microbiol* 2012, **78**:227-235.
This study was one of the first to examine the effects of acaricide application on viral dynamics.

89. vanEngelsdorp D, Meixner MD: **A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them**. *J Invertebr Pathol* 2010, **103 Suppl1**:S80-S95.

90. Nazzi F, Le Conte Y: **Ecology of Varroa destructor, the major ectoparasite of the Western Honey Bee, *Apis mellifera***. *Annu Rev Entomol* 2015 http://dx.doi.org/10.1146/annurev-ento-010715-023731.

91. Boncristiani H, Underwood R, Schwarz R, Evans JD, Pettis J, vanEngelsdorp D: **Direct effect of acaricides on pathogen loads and gene expression levels in honey bees *Apis mellifera***. *J Insect Physiol* 2012, **58**:613-620.

92. •• Kuster RD, Boncristiani HF, Rueppell O: **Immunogene and viral transcript dynamics during parasitic Varroa destructor mite infection of developing honey bee (*Apis mellifera*) pupae**. *J Exp Biol* 2014, **217**:1710-1718.
This is an excellent investigation of the role of mites and virus infection on honey bee immune gene expression.

93. Johnson RM, Dahlgren L, Siegfried BD, Ellis MD: **Acaricide, fungicide and drug interactions in honey bees (*Apis mellifera*)**. *PLoS ONE* 2013, **8**:e54092.

94. Hopwood J, Vaughan M, Shepherd M, Biddinger D, Made E, Black S, Mazzacano C: *Are Neonicotinoids Killing Bees? A Review of Research into the Effects of Neonicotinoid Insecticides on Bee, with Recommendations for Action, 2012*. 2016:. xerces.org, access date February.

95. Commission Implementing Regulation (EU) No 485/2013 [Internet]. 2013, in press.

96. *Schedule for Review of Neonicotinoid Pesticides*. Environmental Protection Agency; 2016:. [Internet] Access date: February.

97. Mattila HR, Rios D, Walker-Sperling VE, Roeselers G, Newton I: **Characterization of the active microbiotas associated with**

honey bees reveals healthier and broader communities when colonies are genetically diverse. *PLoS ONE* 2012, **7**:e32962 http://dx.doi.org/10.1371/journal.pone.0032962.

98. Anderson KE, Carroll MJ, Sheehan T, Mott BM, Maes P, Corby-Harris V: **Hive-stored pollen of honey bees: many lines of evidence are consistent with pollen preservation, not nutrient conversion**. *Mol Ecol* 2014, **23**:5904-5917.

99. Kapheim KM, Rao VD, Yeoman CJ, Wilson BA, White BA, Goldenfeld N, Robinson GE: **Caste-specific differences in hindgut microbial communities of honey bees (*Apis mellifera*)**. *PLoS ONE* 2015, **10**:e0123911.

100. Corby-Harris V, Maes P, Anderson KE: **The bacterial communities associated with honey bee (*Apis mellifera*) foragers**. *PLoS ONE* 2014, **9**:e95056.

101. Pfeiffer JK, Virgin HW: **Viral immunity. Transkingdom control of viral infection and immunity in the mammalian intestine**. *Science* 2016, **351** http://dx.doi.org/10.1126/science.aad5872.

102. Martinez J, Longdon B, Bauer S, Chan Y-S, Miller WJ, Bourtzis K, Teixeira L, Jiggins FM: **Symbionts commonly provide broad spectrum resistance to viruses in insects: a comparative analysis of wolbachia strains**. *PLoS Pathog* 2014:10.

103. Teixeira L, Ferreira A, Ashburner M: **The bacterial symbiont Wolbachia induces resistance to RNA viral infections in *Drosophila melanogaster***. *PLoS Biol* 2008, **6**:e2.

104. Jeyaprakash A, Hoy MA, Allsopp MH: **Bacterial diversity in worker adults of Apis mellifera capensis and Apis mellifera scutellata (Insecta: Hymenoptera) assessed using 16S rRNA sequences**. *J Invertebr Pathol* 2003, **84**:96-103.

105. Pattabhiramaiah M, Brueckner D, Witzel KP, Junier P, Reddy MS: **Prevalence of Wolbachia in the European Honeybee, Apis mellifera carnica**. *World Appl Sci J* 2011, **15**:1503-1506.

106. Corby-Harris V, Snyder LA, Schwan MR, Maes P, McFrederick QS, Anderson KE: **Origin and effect of Alpha 2.2 Acetobacteraceae in honey bee larvae and description of *Parasaccharibacter apium gen*. nov., sp. nov**. *Appl Environ Microbiol* 2014, **80**:7460-7472.

107. Corby-Harris V, Snyder LA, Meador CAD, Naldo R, Mott BM, Anderson KE: ***Parasaccharibacter apium*, gen. nov., sp. nov., improves honey bee (Hymenoptera: Apidae) resistance to Nosema**. *J Econ Entomol* 2016 http://dx.doi.org/10.1093/jee/tow012.

108. Engel P, Vizcaino MI, Crawford JM: **Gut symbionts from distinct hosts exhibit genotoxic activity via divergent colibactin biosynthesis pathways**. *Appl Environ Microbiol* 2015, **81**:1502-1512.

109. Engel P, Bartlett KD, Moran NA: **The bacterium *Frischella perrara* causes scab formation in the gut of its honeybee host**. *MBio* 2015, **6** e00193-15.

110. Evison SEF, Roberts KE, Laurenson L, Pietravalle S, Hui J, Biesmeijer JC, Smith JE, Budge G, Hughes WOH: **Pervasiveness of parasites in pollinators**. *PLoS ONE* 2012, **7**:e30641.